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GRANT TITLE: Macrophage Responses to *B. anthracis*

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ABSTRACT:

Using DNA microarrays, we have established the gene expression patterns of *B. anthracis* cells throughout the entire process of sporulation. Over 2,000 genes displayed growth-phase dependent gene expression, 900 of these corresponding to sporulation-specific gene expression events. In a separate study, we compared 19 strains from the *B. cereus* group to *B. anthracis,* Ames using comparative genome hybridization (CGH). This comparison indicated that horizontal gene acquisition within this group of bacteria, has strongly influenced the evolution of *B. anthracis.* Horizontal acquisition and diversity of gene content was more variable on the virulence plasmids compared to the chromosome. With respect to the chromosome alone, variability was more pronounced in the 1.5 Mb region flanking the replication termini. Several *B. anthracis* specific sequences were identified and represent potentially useful markers for discriminating *B. anthracis* from other related and un-related bacterial species.

KEY WORDS:

Bacillus anthracis, macrophage responses, molecular pathogenesis, DNA microarrays, sporulation

OBJECTIVE:

The overall objectives of this proposal are to develop methods and technologies required for performing comprehensive determination of mRNA and protein expression levels of macrophages in relevant models of experimental anthrax infection and intoxication. We examined the gene expression levels of anthrax during the process of formation of its endospore. Sporulation, is the developmental process through which functional endospore, the contagion of the disease anthrax. Understanding of the anthrax endospore will help in determining its specific properties that make it a robust pathogen and may help predict methods for detection and countermeasures.

The microarray was also used to compare various genomic features of *B. anthracis* to 19 members of the *B. cereus* group, by Comparative Genome Hybridization (CGH). *B. anthracis* is a member of the *B. cereus* group. This closely related clade, which includes *B. cereus* and *B. thuringiensis,* is indistinguishable on the basis of 16S rRNA sequence and can for practical purposes be considered one species. However, the group exhibits substantial diversity in their pathogenic characteristics. While *B. anthracis* is highly virulent to many mammals, *B. cereus* is

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15. SUBJECT TERMS
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an opportunistic pathogen causing food-borne diseases as well as local and systemic infections in man. *B. thuringiensis* is an insect pathogen used widely as a biological pesticide. We exploited the close genotypic relationship of the *B. cereus* group as a means of identifying candidate genes that account for the profound phenotypic properties observed within this group, as it relates to pathogenicity and human virulence.

APPROACH:

We determined the gene expression of *B. anthracis* during sporulation and the protein content of the spore as determined by multidimensional chromatography and tandem mass spectrometry (Liu, et al., 2004). The combined use of these two global approaches provided a means of relating the observed gene expression to the proteome of the spore itself. We compared the RNA expression patterns of twenty samples (15-minute intervals) over a five-hour period corresponding to exponential, non-exponential and stationary phase growth as well as, the entirety of sporulation.

ACCOMPLISHMENTS:

We successfully determined the global gene expression profiles of *B. anthracis* cells as they entered into the developmental phase of sporulation as well as all subsequent events leading up to the final construction of functional (infectious) endospore particles. The data derived from this study was combined with proteomics data generated by the Yates laboratory to draw insights into the gene expression patterns associated with sporulation as it relates to the construction, loading and processing of functional endospores.

CONCLUSIONS:

The dormant spore of *Bacillus anthracis*, the bacterium thought to cause anthrax, is the infectious agent. Upon entry into a host, through inhalation, the spore efficiently infects macrophages where it rapidly germinates within the harsh environment of the phagolysosome. The determination of the gene expression in *B. anthracis* cells undergoing sporulation (induced by nutrient starvation) and of the spore germination, are of fundamental interest. It is known that the onset and progression of sporulation in the closely related *B. subtilis,* involves the sequential activation of a number of sigma factors that mediate and coordinate the temporal expression of the so-called, sporulation genes. In *B. anthracis*, a significant and reproducible change in gene expression was observed for over 3,500 genes (63% of the genome). Just over 2,000 genes displayed growth-phase regulated expression in two or more consecutive time points (>30 minutes). Hierarchical clustering of the expression data revealed a complex set of expression patterns. The gene expression patterns can be represented by five distinct patterns, based on the temporal onset of gene expression changes (activation or repression). These five patterns appear to correspond to biologically meaningful phases as judged by the number of transcripts and transcriptional regulators involved at each phase. Approximately 1,100 genes displayed altered RNA expression within the first two phases, which correspond to cells exiting exponential growth phase and entering stationary phase growth and stationary phase growth just prior to the onset of sporulation. The heterogeneity of transcriptional behavior among genes within these

two phases is substantial especially when compared to the transcript profiles corresponding to ~900 genes observed in the subsequent three phases occurring during sporulation.

Once inside the macrophage, the *B. anthracis* spore enters into a race with the host immune response. Consequently, the spore has become uniquely adapted to germinate and begin vegetative growth rapidly. For this reason and others, it had been assumed that the spore contained proteins to ensure efficient re-entry into a growth phase. Among the 750 proteins identified in the endospore, half are encoded by genes expressed consitutively throughout the entire experimental timecourse. Consistent with prior assumptions, these transcripts encode proteins biased in functional representation, the most over-represented classes being those involved in, protein synthesis, nucleoside/tide biosynthesis and energy metabolism. The remaining half of the endospore proteome is comprised of proteins encoded by genes displaying altered RNA expression in each of the five expression phases in approximately equivalent proportions. It was not anticipated that gene expression changes occurring during the first two growth phases would contribute equally to the endospore as genes differentially expressed during sporulation. It is somewhat surprising that approximately 15% of the spore proteome is derived from strongly repressed genes, an interesting sub-set of which are up-regulated (~75 minutes) as cells enter saturation phase. These genes are subsequently repressed as cells enter into sporulation. This gene expression pattern may suggest that preparations for sporulation may occur prior to the cell's actual commitment to this pathway. It is noteworthy, that less than 20% of the genes displaying sporulation-limited RNA expression, encode proteins present in the endospore. Taken together, the two global analyses support the view that *B. anthracis* sporulation is a highly controlled and complex process. The proteins (raw-materials) and enzymes (tools) required to construct and load the endospore, are expressed in a growth-phase dependent and independent manner. The proteins encoded by genes displaying sporulation-specific expression contribute several functions related to the structure and architecture of the endospore. The function and significance of many proteins differentially expressed during sporulation remain to be determined, however our data suggest that the majority of differential gene expression associated with sporulation is intended to provide proteins required for the processing of the raw materials and the physical construction of the spore.

A comparison of genomic features of *B. anthracis* to 19 members of the *B. cereus* group by comparative genome hybridization (CGH), supports the idea that major portions of the pXO1 plasmid were present in a common ancestor of *B. anthracis* and *B. cereus*, prior to the former's evolution into a mammalian pathogen and that gene transfer events have occurred, particularly involving the pXO1 virulence plasmid. Our data suggest that the evolution of virulence in *B. anthracis* has been driven by exchanges of genetic elements among its closest relatives.

SIGNIFICANCE:

Anthrax research has become increasingly important since anthrax is a choice pathogen for people motivated to acts of biowarfare. Anthrax expresses its virulence genes in an organized, temporally-regulated and tissue-specific manner. Especially important are the events surrounding anthrax-macrophage interactions. The contents of the spore are a particularly important piece of information, since it is primarily through the "stockpile" of small molecules and proteins stored in the endospore that allow its efficient re-entry into a vegetative growth

phase upon contact with host macrophages. Therefore, it is possible develop a precise understanding of basic molecular tenets of the infectious cycle and host responses by tracking gene and protein expression patterns. This will allow for discovery of new anthrax virulence factors and designation of specific molecular targets for interventions and antibacterial drug studies and the development of novel rapid diagnostics. The genomic and proteomic data and the comparison of the two, has broad application for research of many infectious diseases.

The CGH experiments allowed us to successfully identify several large regions (6) that appear to be *anthracis*-specific chromosomal insertions, thereby provide useful signature sequences through which diagnostic devices may robustly discriminate *B. anthracis* from other related and unrelated bacterial species. Our data suggest that the evolution of virulence in *B. anthracis* has been driven by exchanges of genetic elements among its closest relatives.

PATENT INFORMATION: none

AWARD INFORMATION: none

PUBLICATIONS AND ABSTRACTS:

1. Liu, H., N.H. Bergman, B. Thomason, S. Shallom, A. Hazen, J. Crossno, D.A. Rasko, J. Ravel, T.D. Read, **S.N. Peterson**, J. Yates III and P.C. Hanna (2004). Formation and composition of the Bacillus anthracis endospore. *J. Bacteriol* **186**:164-78.

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